

The Cancer Target Discovery and Development Network*

Small-molecule drug discovery was originally a compound-based activity. The process begins with the discovery of a biologically active compound, often a naturally occurring small molecule. The next step involves the identification of a disease that may benefit from treatment with the compound, followed by optimization and development of the eventual drug (or drugs through synthetic modifications). Penicillin is an early example of a drug that arose from this approach. Despite many advances in drug discovery in the intervening decades, compound-based drug discovery is still common today. Rapamycin (Rapamune, sirolimus), for instance, was discovered as a secondary metabolite of a *Streptomyces* strain and was explored without success as an antifungal agent before emerging as an effective immunosuppressive agent. Synthetic derivatives of rapamycin have now been approved or are being investigated as therapeutics in cancer (Torisel, temsirolimus; Afinitor, everolimus; ridaforolimus) and in other diseases.

Advances in human genetics suggest that a third approach—patient-based drug

Probe acquired dependencies via RNA^(CU, DFCI, UT)

Cancer genomics

Probe acquired dependencies via proteins^(BI, UT)

(Small-molecule probe set)

Determine relevance (STK33; TBK1)^(BI, DFCI, UT)

Small-molecule probes^(BI)

Determine relevance (STAT3; C/EBP in GBM)^(BI, CU)

Cancer genomics-based mouse models^(CSH, DFCI)

Probe acquired dependencies via network analyses^(CU)

Cancer patients

Figure 1 The NCI's Cancer Target Discovery and Development (CTD²) Network aims to relate the genetic features of cancers to acquired cancer dependencies and to identify small molecules that target the dependencies. The centers where the approach is being undertaken are abbreviated in parentheses: BI, Broad Institute of Harvard and MIT; CSH, Cold Spring Harbor Laboratory; CU, Columbia University; DFCI, Dana-Farber Cancer Institute; and UT, University of Texas, Southwestern Medical Center at Dallas.

Although heritable disorders and infectious diseases are the subject of intensive

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not without additional challenges for payers, patients and healthcare providers^{2,4}.

The National Cancer Institute's approach

To pursue this path comprehensively and prospectively, the US National Cancer Institute (NCI) created the Cancer Target Discovery and Development (CTD²) Network (<http://ocg.cancer.gov/programs/tddn.asp>). The Network currently comprises five interacting centers (Fig. 1). The mission of the CTD² Network is to decode cancer genotypes so as to read out acquired pathway and oncogene addictions of the specific tumor subtypes and to identify small molecules that target these dependencies. The Network builds on the data and insights gained from The Cancer Genome Atlas, Therapeutically Applicable Research to Generate Effective Treatments initiative and other cancer genomic efforts that are systematically cataloging the genetic and epigenetic alterations of specific cancers (e.g., mutational status and changes in gene expression, DNA methylation and chromosomal segment copy numbers). The CTD² Network is probing the consequences of these alterations on the dependencies or co-dependencies different cancers have on specific oncogenes or their interacting genes (that is, 'oncogene addiction' and 'nononcogene co-dependencies')⁵. Cataloging these Achilles' heels and linking them to the causal genetic alterations will be critically important for therapies that are personalized to individuals, including combination therapies aimed at targeting many such dependencies at once. It will also be important for anticipating resistance mechanisms and identifying clinical biomarkers.

The CTD² Network is currently taking five integrated approaches to determine the targets and processes upon which defined cancer genotypes become dependent. First, techniques that enable the systematic under- or overexpression of selected mRNA transcripts are being used to identify candidate genes. Second, computational network analyses are being performed on cancer genomic data sets to reveal critical master regulatory hubs in the circuitries of cancers, that act as integrators of the complex spectrum of genetic alterations that determine specific tumor subtypes. Third, a small-molecule probe set has been assembled, having members that modulate the activity of defined proteins and pathways that constitute candidate tumor dependencies. These compounds are being tested in many genomically characterized cancer cell lines, and small-molecule sensitivities are thus being correlated to the genetic features of the cancer cells. (In each of these three approaches, the CTD² Network measures the

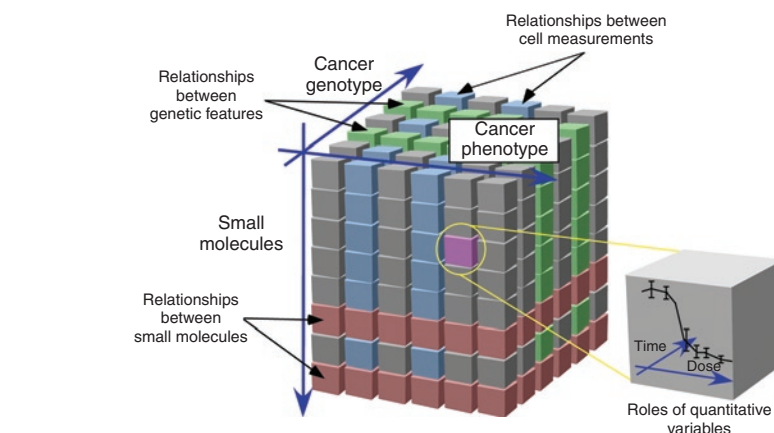


Figure 2 Conceptual image of a matrix of data relating cancer genotype, cancer phenotype and sensitivity to highly specific small-molecule modulators of cancer-relevant proteins. The CTD² Network is performing quantitative cellular measurements using small molecules (both with and without a knowledge of their targets) and genetically characterized cancer cell lines (copy number variation, mutational status and gene expression). Computational analyses are being performed that correlate the pattern of sensitivity with the genetic features of the cancer cell lines^{9–11}. These analyses yield hypotheses for cancer genotype–drug efficacy relationships that can be tested *in vitro* and *in vivo* using systems developed within the CTD² Network.

fitness of cancer cells having defined genetic features following targeted perturbations.) Fourth, simultaneously, probe-development projects are being undertaken to yield novel small molecules that modulate the functions of cancer therapeutic targets revealed by these approaches. Finally, the consequences of these and other agents that interfere with gene function are being, or will be, tested in, for example, mouse models of cancer having genetic alterations that closely mimic the patient-derived cancers (Fig. 1).

Probing acquired dependencies using RNA

The CTD² Network is exploiting the extraordinary advances in modulating gene function using RNA interference-based knockdown or RNA overexpression methods. Three examples illustrate the principles behind this approach to identifying acquired somatic genotype-specific dependencies.

The CTD² center at the University of Texas Southwestern Medical Center at Dallas is screening genomic small inhibitory (si) RNA libraries against a large panel of non-small cell lung cancer cell (NSCLC) lines derived from human tumors to identify, as a particular NSCLC subtype or clade, siRNAs that are lethal only to cancers that share a similar cancer genotype⁶. Clade-specific lethal siRNAs are being used to identify metabolic vulnerabilities that occur in a particular cancer subtype, vulnerabilities that might be exploited for developing genetically matched anti-cancer therapeutics.

The CTD² center at the Dana-Farber Cancer Institute in Boston is screening

short-hairpin (sh)RNA libraries to identify different types of cancer vulnerabilities. For example, in a screen of 20 human cancer cell lines, Barbie *et al.*⁷ have looked for kinases selectively required for cell survival that depend on oncogenic KRAS and found that, second only to KRAS itself, the noncanonical kinase TBK1 was a synthetic lethal partner.

At Columbia University in New York, the CTD² center is using pooled shRNA libraries to complement the computational analysis of master regulators of high-grade glioma subtypes and of glucocorticoid resistance in T-cell acute lymphoblastic leukemia.

Probing acquired dependencies by network analyses

Context-specific regulatory networks of the tumor cell are being assembled and interrogated computationally to reveal otherwise cryptic master regulator proteins whose gain or loss is necessary and sufficient for tumor initiation or progression. These proteins are emerging as master 'integrators' of a spectrum of genetic and epigenetic alterations contributing to the malignant phenotype and thus provide promising novel biomarkers as well as targets for therapeutic intervention (Fig. 2).

For instance, at the CTD² center at Columbia, C/EBP and STAT3 were recently identified as synergistic master regulators of the mesenchymal subtype of glioblastoma by computational analysis of a regulatory network dissected from a large collection of gene expression profiles of human high-grade gliomas⁸. Validation was achieved by two

experimental approaches: shRNA-mediated silencing of these two genes reduced tumor aggressiveness in orthotopic xenografts and co-ectopic expression reprogrammed murine neural stem cells along an aberrant mesenchymal lineage.

Probing acquired dependencies by modulating proteins

The dramatic clinical consequences of linking genetic features of cancers to drug efficacies, including response rates of >80%, are well known, yet these advances today benefit <1% of those suffering from cancer³. The CTD² centers at the Broad Institute in Cambridge, Massachusetts, and the University of Texas, Southwestern Medical Center, are relating the genetic features of cancers to small-molecule probes or drug efficacies broadly. The CTD² Network is extending earlier efforts^{9–11} in several ways: first, it is assembling and synthesizing highly specific small molecules (currently a collection of 225 probes and drugs) that target a wide range of proteins and that exploit advances in probe discovery^{12,13}; second, it is creating small-molecule screening collections with novel chemical properties; third, it is making quantitative cellular measurements in a wide range of human cancer cell lines treated with the compounds; and fourth, it is identifying the genetic features in these cells that correlate with sensitivities of the small-molecule probes or drugs.

The CTD² Network is studying the novel compounds it identifies using cell lines whose genomic features (e.g., copy number, mutation or expression) have been richly characterized and parallel many of the changes found in human cancers^{14,15} (although not without exception^{16,17}). The intent of this effort is to identify (i) therapeutic targets of cancers linked to specific genetic features associated with cancers¹⁰; (ii) combinations of targets that, by using guided combination therapy, yield high rates of durable responses; and (iii) potential resistance mechanisms associated with such targets¹⁸. The resulting data and resources will be publicly available through

the project's web site at the end of this year (<http://ctd2.nci.nih.gov>).

Probe-development projects for novel cancer targets

The CTD² Network also aims to accelerate the development of genetically matched cancer drugs by discovering novel small-molecule probes of candidate cancer targets not yet modulated by small molecules. The goal is to identify these gaps and to undertake collaborative probe-development projects involving high-throughput screening, follow-up and medicinal chemistry and biology, and mechanism-of-action studies. Advances in the science of probe discovery, especially in fundamental synthetic chemistry, the culturing and co-culturing of cells using conditions closer to natural physiological environments, and in small-molecule assay development, have enabled the discovery of compounds that modulate challenging cancer-relevant targets and processes^{12,13}.

CTD² investigators are especially interested in projects involving targets such as transcription factors and processes such as gene regulation and cellular differentiation. For example, small-molecule probe-development projects are underway involving both transcription factors (including STAT3, C/EBP (β and δ)⁸ and MYC) and chromatin-modifying enzymes (including histone methyltransferases and histone demethylases) that have been identified from genomic studies of cancer.

Probing genetic alterations in mouse models of human cancers

Genomic characterization of human cancers has revealed many genes that are altered. Transgenic or knockout mice that contain germline alterations in the candidate cancer gene can be used to assess oncogenic function. However, their generation and analysis precludes high-throughput evaluation of mutated genes. Transplantable mouse models offer the advantage of speed because genetic lesions are introduced into stem or progenitor cells that are then transplanted

into recipient animals. Such models exist for a number of cancer types, including lymphoma, glioblastoma and carcinomas of the liver^{19–21}. These models can be used to screen large numbers of genes for oncogenicity and acquired dependencies²² and to determine the efficacy of small-molecule probes that have been optimized for animal testing.

Conclusions

The CTD² Network was formed by the NCI to serve as a link in the overall effort to discover safe and effective patient-based cancer drugs and to facilitate their clinical development through the identification of the genetic features of human cancers that predict drug efficacy, resistance mechanisms and clinical biomarkers. The Network aims to relate these features to their unique dependencies and to identify small molecules that target them, even when this entails hard-to-drug targets and processes—an empirical path that begins and ends with cancer patients.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

- Weinstein, I.B. & Joe, A.K. *Nat. Clin. Pract. Oncol.* **3**, 448–457 (2006).
- Aggarwal, S. *Nat. Rev. Drug Discov.* **9**, 427–428 (2010).
- Thompson, C.B. *Cell* **138**, 1051–1054 (2009).
- Bach, P.B. *N. Engl. J. Med.* **360**, 626–633 (2009).
- Luo, J., Solimini, N.L. & Elledge, S.J. *Cell* **136**, 823–837 (2009).
- Whitehurst, A.W. *et al. Nature* **446**, 815–819 (2007).
- Barbie, D.A. *et al. Nature* **462**, 108–112 (2009).
- Carro, M.S. *et al. Nature* **463**, 318–325 (2010).
- Deininger, M.W., Goldman, J.M., Lydon, N. & Melo, J.V. *Blood* **90**, 3691–3698 (1997).
- McDermott, U. *et al. Proc. Natl. Acad. Sci. USA* **104**, 19936–19941 (2007).
- Ramanathan, A., Wang, C. & Schreiber, S.L. *Proc. Natl. Acad. Sci. USA* **102**, 5992–5997 (2005).
- Frye, S.V. *Nat. Chem. Biol.* **6**, 159–161 (2010).
- Workman, P. & Collins, I. *Chem. Biol.* **17**, 561–577 (2010).
- Lin, W.M. *et al. Cancer Res.* **68**, 664–673 (2008).
- Sos, M.L. *et al. J. Clin. Invest.* **119**, 1727–1740 (2009).
- Lee, J. *et al. Cancer Cell* **9**, 391–403 (2006).
- van Staveren, W.C. *et al. Biochim. Biophys. Acta* **1795**, 92–103 (2009).
- Turke, A.B. *et al. Cancer Cell* **17**, 77–88 (2010).
- Zender, L. *et al. Cell* **125**, 1253–1267 (2006).
- Zheng, H. *et al. Nature* **455**, 1129–1133 (2008).
- Zuber, J. *et al. Genes Dev.* **23**, 877–889 (2009).
- Zender, L. *et al. Cell* **135**, 852–864 (2008).

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